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INCREASED ACTIVITY OF CONSTITUTIVE NITRIC OXYDE SYNTHASE IN THE PORCINE CILIARY BODY

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Purpose. The conversion of L-arginine into L-citrulline by nitric oxide synthase (NOS) leads to nitric oxide formation. Two major NOS isoforms exist, a Ca^{++}-dependent NOS (cNOS) and a Ca^{++}-independent NOS (iNOS). The cNOS is constitutive of many cells while the iNOS is not expressed in normal conditions but only induced after the stimulation by different factors (endotoxins, cytokines, etc...). This study addresses the spontaneous cNOS and iNOS activity in porcine ocular tissues.

Methods. The cornea, the iris, the retina, and the ciliary body were dissected free and stored into liquid nitrogen. The NOS activity was assayed by measuring the transformation of L-[U-^{14}C]-arginine into L-[U-^{14}C]-citrulline, 1) in absence or in presence of the Ca^{++} chelator, ethylene glycol tetra acetic acid (EGTA), and 2) in presence of EGTA and the NOS-inhibitor, N^{G}-nitro-L-arginine methyl ester (L-NAME).

Results. In the cornea ($n = 7$; $p < 0.05$), the iris ($n = 5$; $p < 0.05$), and the retina ($n = 6$; $p < 0.005$) there was a high cNOS activity. In these tissues, no significant iNOS activity could be detected. On the opposite, there was no cNOS, but a marked iNOS activity ($n = 5$, $p < 0.005$) in the ciliary body.

Conclusions. These results demonstrate for the first time the presence in a tissue of an apparently spontaneous iNOS activity. The physiological implications of this observation for the regulation of the intraocular pressure, the accommodation mechanisms, or the ocular immune response need to be further investigated.

Supported by the Schwickert Foundation and the SNSRF (grant 32-42564.94).

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EFFECTS OF INTRAOCULAR INJECTION OF NO DONNORS IN THE RABBIT.

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Purpose: Nitric Oxyde (NO) is synthesized by two types of NO synthases: A constitutive isoforme (cNOS) which acivity is calcium-calmodulin dependent, and an inducible one (iNOS), regulated by cytokines and stimulated by lipopolysaccharide (LPS). NOSc activity has been demonstrated in the ciliary body, in the choroid, and in the retina (amacrines cells, RPE and ROS). NO could be implicated in the regulation of intra-ocular pressure (increase or decrease) and of choroidal blood flow, in retinal neurotransmission and in inflammatory retinal process. Our purpose was to evaluate the effects and the kinetic of action of NO donnors in vivo, alone or in combination with other free radicals, in the rabbit eye.

Material and methods: High concentrated 3-morpholonosynonimide SIN-1) and S-Nitroso-N-acetyl-D,Lpenicillamine (SNAP) (10µl, 5-10 mM) solutions were injected intra-camerally and in the vitreous. Xanthine oxydase (XaO 0.5 and 1 mU) was also injected either alone or in combination with SNAP. Slit-lamp examination and photographs were performed at regular intervals for 1 month. Examination of the posterior segment by indirect ophthalmoscopy and mesure of the intraocular pressure (IOP) were also performed. Nitrites levels, reflecting NO release was evaluated in aqueous humor and in the vitreous by Griess reaction at different periods after injection. Ocular tissues were histologically examined.

Results: No inflammation was observed as a result of NO donnors injection, except a transitory conjunctival vasodilatation. A drastic drop of IOP occured within 15 minutes after intravitreal injection. The decrease of pressure was correlated to the production of nitrite in the vitreous. No histological lesion occured until 1 month after injection. Inflammation due to XaO was increased by SNAP injection (proteins and inflammatory cells).

Conclusions: NO alone did not induce any inflammation but it potentiated the inflammation generated by other free radicals. IOP drop is correlated to nitrite production after intravitreal injection of NO donnors.

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HISTOCHEMICAL LOCALIZATION OF NADPH DIAPHORASE REACTIVITY IN VASCULAR ENDOTHELIAL CELLS IN THE HUMAN UVEAL TRACT

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Purpose. Previous studies have demostrated a rich population of NADPH diaphorase and eNOS positive neurons and fibers in the choroid of humans, rats and monkeys (Bergua 1993, Flügel 1994) which can exert vasodilator actions on local vasculature. In addition, endothelial nitric oxide (eNO) play a important role in tonic control of uveal vessels (Zagvazdin, 1995). The aim of the present study was investigated the endothelial nitrergic localization within vascular endothelial cells of the human uveal tract using the NADPH-diaphorase method. **Methods.** Human uveal tissue was obtained from 6 human donor eyes (50-72 yr) for corneal transplantation, at a postmortem time of 5-20 h. Uveal tissue were fixed in 4% paraformaldyde/0.1% glutaraldehyde in 0.1M cacodylate buffer (pH 7.4), for 2-4 hours at 4 C. Then, choroids, ciliary bodies and iris were conserved in 10% saccharose buffer for 24 to 72 hours at 4 C. Choroids and iris were stained as whole flat mounts. 14-µm thick ciliary bodies frozen sections were also performed. Histochemical staining for NADPH diaphorase was done by incubating specimens in 0.1 M phosphat buffer (pH 7.4) containing 1 mg/ml ß NADPH (Biomed), 0.1 mg/ml Nitro Blue Tetrazolium (Roth) and 0.3% Triton X 100 for 1-12 hours at 37 C.

Results. Human uveal vascular endothelial cells revealed positive NADPH diaphorase activity, which was detected by the presence of punctate blue staining in the cytoplasm. No significant difference in distribution pattern, or area density was seen amongst small and large vessels in either choroid, ciliary body or iris. Ganglion cells in the choroid and ciliary body also exhibited NADPH diaphorase reactivity.

Conclusions. The present investigation demostrates NADPH diaphorase reactivity in vascular endothelial cells of the human uveal tract suggesting a possible vascular nitrergic control mechanism of this structure.

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MOBILISATION OF INTRACELLULAR CALCIUM IN CULTURED, NON-TRANSFORMED, BOVINE CILIARY EPITHELIAL CELLS.

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Purpose Virtually all types of cell (including secretory) are activated by a rise in the intracellular concentration of calcium ions (Ca^{2+}_i). We have measured the ability of drugs to produce transient changes in the Ca^{2+}_i of cultured non-transformed ciliary epithelial cells in an effort to elucidate the fundamental mechanisms which may be involved in controlling aqueous humour formation.

Methods Ciliary epithelial cells were cultured from fresh bovine eyes (Shahidullah et al., 1995). First passage cells (largely pigmented epithelium) were grown to confluence on glass coverslips. Ca^{2+}_i was measured by standard ratiometric methods using the fluorescent probe Fura-2.

Results Ca^{2+}_i levels in cultured ciliary epithelium bathed in Krebs solution were 138±2nM (mean ± S.E.M., n=103). Addition of acetylcholine or adrenaline (1-1000µM final concentration) produced no change in Ca^{2+}_i. In contrast, addition of ATP or UTP (1-100µM final concentration) caused a rise in Ca^{2+}_i (peak within 30sec), which was dose-dependent. Exposure to 100µM ATP significantly increased Ca^{2+}_i to 364±22nM (n=25). These responses showed rapid desensitisation and cross-desensitisation between ATP and UTP. In Ca^{2+}-free Krebs' solution (containing EGTA, 0.5mM), 100µM ATP produced a rise in Ca^{2+}_i to 295±27nM (n=12).

Conclusions Absence of any response to acetylcholine or adrenaline is in contrast to a report of their action in rabbit non-pigmented epithelium (Ohuchi et al., 1992). Increases in Ca^{2+}_i by ATP and UTP suggest the presence on bovine ciliary epithelium of purinergic receptors of the P_{2U} subtype, found in a wide variety of cells, including secretory. Persistence of the response in Ca^{2+}-free medium indicates that ATP releases Ca^{2+} from an intracellular site. This system offers the opportunity to study effects on Ca^{2+}_i of drugs which are known to alter aqueous humour formation.

References Ohuchi,T. et al. (1992). Invest. Ophthalmol. Vis. Sci. 33, 1696.
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